AWARD NUMBER: W81XWH-15-1-0683

TITLE: Diagnosis of Late-Stage, Early-Onset, Small-Fiber Polyneuropathy

PRINCIPAL INVESTIGATOR: Anne Louise Oaklander MD, PhD

CONTRACTING ORGANIZATION: Massachusetts General Hospital
Boston, MA 02114

REPORT DATE: October 2016

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
October 2016	Annual	30 Sep 2015 - 29 Sep 2016

4. TITLE AND SUBTITLE

Diagnosis of Late-Stage, Early-Onset, Small-Fiber Polyneuropathy

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-15-1-0683

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Anne Louise Oaklander MD, PhD
Max M. Klein PhD

E-Mail: aloaklander@mgh.harvard.edu

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

Massachusetts General Hospital
Boston, MA 02114

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

Our prior award generated evidence that some syndromes involving unexplained chronic multisymptom illness (CMI) and chronic widespread pain (CWP), including Gulf War Illness (GWI), may involve small-fiber polyneuropathy (SFPN). Objective diagnostic measures suggest that some veterans with GWI may have longstanding, persistent early-onset SFPN. We are developing and evaluating generally applicable tools that can better screen and test Veterans with GWI for such long-standing, early-onset SFPN. These include simple screening tools such as targeted questionnaires and detection of abnormal sweating, pupil size and reactivity. Once diagnosed, we plan to develop and apply DNA sequencing panels to identify polymorphisms that may convey risk for developing SFPN. This project, which expands on our previous findings, is in collaboration with the Veterans Biomedical Research Institute at the East Orange NJ Veterans Administration Hospital. This report covers first year progress.

15. SUBJECT TERMS

Neuropathy, Gulf War Illness, chronic widespread pain, chronic multisymptom illness, small-fiber polyneuropathy, case definition

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
U	U	U	UU	10	19b. TELEPHONE NUMBER *(include area code)*

Table of Contents

Page

1. INTRODUCTION:

Small nerve fibers, which are the long, small-diameter, unmyelinated C-fibers or thinly myelinated A-delta fibers that sense pain and regulate the function of internal organs and tissues, are susceptible to malfunction and degeneration at their farthest ends if their oxygen, nutrient, or energy supply is compromised, resulting in small-fiber polyneuropathy (SFPN). SFPN symptoms include unexplained chronic widespread pain (CWP) and chronic multisymptom illness (CMI), including cardiovascular, gastrointestinal, microvascular, and/or disordered sweating, which contributes to heat and exercise intolerance and fatigue, similar to Gulf War Illness. Given these non-specific symptoms, objective testing is recommended for SFPN diagnosis. Our prior research suggests that SFPN is prevalent in CWP and CMI syndromes [1]. We additionally discovered SFPN that affects adolescents and adults [2]. This early-onset SFPN usually begins in adolescence or early adulthood but can linger to cause CWP and CMI for decades, like Gulf War Illness. Importantly, some causes of early-onset SFPN can be treated and even cured. Our previous preliminary data show that among 38 Gulf War veterans and 41 matched controls, 49% of veterans had objective evidence of SFPN vs. 12% of controls [3]. We now propose to build on our prior findings in collaboration with the East Orange VA Hospital to develop targeted questionnaires and examination forms that we will perfect and validate using well-characterized controls and veterans. We will assess the utility of a simplified sweat test (Sudoscan™) and measurement of pupil size and reactivity. We also plan to develop a genetic sequencing panel capable of uncovering genetic markers of SFPN. This has potential as a screening tool for enlistees who do not have SFPN, but may have a genetic predisposition to develop it later in life or express it in response to environmental triggers encountered by warfighters, such as neurotoxic exposures.

2. KEYWORDS:

Neuropathy, Gulf War Illness, chronic widespread pain, chronic multisymptom illness, small-fiber polyneuropathy, case definition

3. ACCOMPLISHMENTS:

What were the major goals of the project?

Objective/Hypothesis: To clinically translate our high-impact discoveries from our prior GW093049 award into generally applicable tools that providers throughout the US can use to better diagnose and treat local veterans with GWI.

Specific Aims:

Specific Aim 1: To develop and evaluate screening tools for diagnosis and monitoring of longstanding early onset SFPN, specifically a patient-reported symptom questionnaire and a standardized examination for medical personnel. These will be validated in samples of ≥ 100 well-characterized GW-veterans and civilians.

Specific Aim 2: To develop and evaluate simple biotechnology devices for diagnosing and monitoring longstanding early onset SFPN based on detection of abnormal sweating and pupil size and reactivity. These will be validated in large, well-characterized samples of GW-

veterans and demographically matched civilians.

Specific Aim 3: To develop and evaluate tools for identifying gene polymorphisms that convey risk for early onset SFPN: A panel of sequencing tests will be developed using DNA samples from 50 veterans whose GW illness has been recharacterized as longstanding early onset SFPN.

What was accomplished under these goals?

This being the first year of this study, accomplishments largely centered on developing the tools and methodologies to perform the human subject studies scheduled to begin in the second year of the study.

All Aims:

Human Research Approval: Develop protocol, obtain IRB and Human Research Protection Office approvals (Months 1-3):

> We amended our existing research protocol to include this study, and added a Patient Information sheet to inform all participants that the USAMRMC is the sponsor and has rights regarding access to the study data. We also fulfilled DoD HRPO requirements by adding an independent Research Monitor to the protocol. Subsequently we successfully gained local IRB Continuing Review approval of our protocol which was again approved by HRPO.

Recruitment: Search DMDC data request system, Research Patient Data Registry (RPDR), cross-check with electronic medical records, advertisement, contact with patient advocacy groups and Veterans' Service Organizations (VSOs), leverage other ongoing studies (Months 3-30):

> We discussed recruitment techniques advantageous to our two sites with Partnering PI, Dr. Jorge Serrador, using the methods described above and also using the resources unique to the WRIISC that are complementary to MGH's RPDR. We also discussed recruitment strategies with the PI of GWIRP award GW130100, Dr. Marco Loggia, also located at MGH, to enhance enrollment by leveraging our mutual resources.

Specific Aim 1: Pre-field testing of questionnaires and exam forms, develop improved versions (Months 3-12), Administer/validate questionnaires/forms (Months 12-30):

> PI, Dr. Oaklander, took formal instruction in "Applied Methods in Designing & Conducting Health Surveys" and improved the content of the symptom questionnaire under development. We continued to pre-field test the symptom questionnaire followed by cognitive debriefing where subjects were interviewed about their interpretation of the questionnaire. These patients so far had already had skin biopsies and/or autonomic function testing done clinically. This will be followed by initial validation, during which we will look for internal consistency and test re-test reliability. We also improved our standardized examination form.

Questionnaire data from 85 "gold standard" patients with objective evidence of neuropathy (abnormal skin biopsy or autonomic function test) were analyzed. Preliminary results indicate that SFPN patients experience several prevalent patient-reported symptoms not classically associated with SFPN, including fatigue (100% of respondents), reduced endurance (98%), cognitive concerns (88%), and headache (72%). Internal consistency and test-retest reliability of the questionnaire were excellent.

Specific Aim 2: Train WRIISC personnel on skin biopsy technique and shipping to MGH for analysis (Months 2-4):

We developed a process for providing the Partnering site fixative and mailing supplies for obtaining and then sending skin biopsies to our site for processing and interpretation. The Partnering PI also identified suitable personnel at the Partnering site to perform skin biopsies. Next we will ensure that Partnering personnel are properly trained in the skin biopsy technique.

Specific Aim 2: Study SFPN patients and controls that are not already well-characterized with AFT and skin biopsy (Months 8-30):

We met with Partnering site personnel to provide in-person training on autonomic function test (AFT) so both sites will perform equivalent tests on respective study subjects.

Specific Aim 2: Perform Sudoscan and pupillometry (Months 8-30):

We purchased a Sudoscan™ (ImpetoMedical, Inc., San Diego, CA) galvanic skin response measurement device. We had earlier performed pilot studies using a rented Sudoscan to gain experience and to demonstrate proof-of-concept on well-characterized subjects that it could detect sweating abnormalities due to small-fiber neuropathy.

Partnering PI, Dr. Serrador, has obtained equipment for pupillometry and is integrating the components so they can perform to the study specifications. Once successful, we will proceed to purchase the same equipment and the Partnering PI will configure it for this study.

Specific Aim 3: Blood draw for genetic material (Months 3-24):

We developed a process for obtaining and storing serum for subsequent testing of genetic material. We have been discussing the appropriate genetic sequencing panels with the MGH Center for Human Genetic Research.

What opportunities for training and professional development has the project provided?

Nothing to report. This project is not intended to provide training opportunities. Nonetheless, personnel will gain additional clinical and research skills through their participation.

How were the results disseminated to communities of interest?

Nothing to report. Results thus far are only preliminary and are intended to guide the establishment of the study tools and parameters.

What do you plan to do during the next reporting period to accomplish the goals?

We expect to finalize the symptom questionnaire and complete configuration of the pupillometry apparatus, after which we will have available the full suite of diagnostic instruments for this study. Once both sites are trained in all the study instruments, we will recruit the appropriate subject populations for study.

4. IMPACT:

What was the impact on the development of the principal discipline(s) of the project?

Nothing to report. We expect that the future impact on Veterans' health will be on improving the diagnosis of Gulf War Illness by focusing on signs and symptoms of small-fiber polyneuropathy, and the development of simpler, faster methods that promote early diagnosis.

What was the impact on other disciplines?

Nothing to report.

What was the impact on technology transfer?

Nothing to report.

What was the impact on society beyond science and technology?

As described above, Veterans' health and, by extension, public health will be improved by the methods developed under this project.

5. CHANGES/PROBLEMS:

Changes in approach and reasons for change

There have been no changes in our approach, nor are any changes anticipated.

Actual or anticipated problems or delays and actions or plans to resolve them

Nothing to report.

Changes that had a significant impact on expenditures

Nothing to report.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Nothing to report.

Significant changes in use or care of human subjects

Nothing to report.

Significant changes in use or care of vertebrate animals.

Not applicable.

Significant changes in use of biohazards and/or select agents

Not applicable.

6. PRODUCTS:

Nothing to report. This study is still in its early stages.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name:	Anne Louise Oaklander MD, PhD
Project Role:	PI
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	1
Contribution to Project:	Dr. Oaklander identified and recruited patients, further developed and oversaw pre-field testing and validation of the symptom questionnaire and administered the exam form.
Funding Support:	No other funding support was used to conduct the work under this award.

Name:	Max Klein PhD
Project Role:	Co-Investigator
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	3
Contribution to Project:	Dr. Klein generated and submitted the necessary paperwork for the research protocol to the local IRB and to HRPO, and obtained HRPO approval. He reviewed records of diagnostic skin biopsy and autonomic function tests. He investigated recruitment databases and developed additional recruitment strategies.
Funding Support:	No other funding support was used to conduct the work under this award.

Name:	Stephanie Ortiz BS (replaced Kate O'Neil BS)
Project Role:	Clinical Studies Coordinator/Research Assistant
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	4
Contribution to Project:	Ms. Ortiz assisted with maintaining IRB (and HRPO) documentation. She assisted with recruiting patients and administering the questionnaire and exam form. She managed data collection for pre-field testing.
Funding Support:	No other funding support was used to conduct the work under this award.

Name:	Heather Downs BS
Project Role:	Histotechnologist
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	1
Contribution to Project:	Ms. Downs processed administrative activities related to this study. She assisted with recruiting patients, managed the master skin biopsy database, and provided the process for fixing and sending skin biopsies to our laboratory for analysis. She also maintained the laboratory supplies necessary for this study.
Funding Support:	No other funding support was used to conduct the work under this award.

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

There are no changes to report that impact personnel effort on this project.

What other organizations were involved as partners?

This is a collaborative award, with the Veterans Biomedical Research Institute at the East Orange, NJ Veterans Administration Hospital as the Partnering organization. The Partnering organization is separately funded, and their progress is described in an independent report.

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: As described above, the Partnering site is submitting an independent report

9. REFERENCES

1. Oaklander AL, Herzog ZD, Downs HM, Klein MM. Objective evidence that small-fiber polyneuropathy underlies some illnesses currently labeled as fibromyalgia. *Pain* 2013; 154:2310-2316.
2. Oaklander AL and Klein MM. Evidence of small-fiber polyneuropathy in unexplained, juvenile-onset, widespread pain syndromes. *Pediatrics* 2013;131:e1091-e1100.
3. Oaklander AL and Klein MM. Undiagnosed Small-Fiber Polyneuropathy: Is it a Component of Gulf War Illness? Final Technical Report GW093049, ADA613891, Sept 2014.

10. ACRONYMS AND ABBREVIATIONS

AFT Autonomic function test
CMI Chronic multisymptom illness
CWP Chronic widespread pain
DNA Deoxyribonucleic Acid
DOD Department of Defense
GWI Gulf War Illness
GWIRP Gulf War Illness Research Program
HRPO Human Research Protections Office
IRB Institutional Review Board
MGH Massachusetts General Hospital
PI Principal Investigator
REDCap Research Electronic Data Capture platform
RPDR Research Patient Data Registry
SFPN Small-fiber polyneuropathy
USAMRMC US Army Medical Research and Materiel Command
VA Veterans Administration
VSO Veterans' Service Organization
WRIISC War-Related Illness and Injury Study Center